

What is claimed is:

1. An isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:76 and a nucleic acid molecule encoding a protein comprising amino acid sequence SEQ ID NO:74.
2. An isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a nucleic acid molecule having a nucleic acid sequence encoding a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:31, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:68, SEQ ID NO:73 and SEQ ID NO:74.
3. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73 and SEQ ID NO:74.

NO:71, SEQ ID NO:72, SEQ ID NO:76 a nucleic acid molecule encoding a protein comprising amino acid sequence SEQ ID NO:74; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

- 5       4. An isolated protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid molecule selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:22, SEQ ID  
10      NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:52, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:71; and a nucleic acid molecule encoding a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55 and  
15      SEQ ID NO:74.

- 5       5. An isolated flea protein selected from the group consisting of: a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:31, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID  
20      NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:68, SEQ ID NO:73 and SEQ ID NO:74; and a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein comprising any of said amino acid sequences.

- 6       6. A formulation of flea carboxylesterase proteins, wherein said proteins, when submitted to 14% Tris-glycine SDS-PAGE, comprise a fractionation profile as depicted in Fig. 3, wherein said proteins have carboxylesterase activity.

- 7       7. A formulation of flea carboxylesterase proteins, wherein said proteins, when submitted to IEF-PAGE, comprise a fractionation profile as depicted in Fig. 4 in a lane selected from the group consisting of lane 3, lane 4, lane 5, lane 6 and lane 7, wherein said proteins have carboxylesterase activity.

8. An isolated flea protein that hydrolyzes  $\alpha$ -naphthyl acetate to produce  $\alpha$ -naphthol, when said protein is incubated in the presence of  $\alpha$ -naphthyl acetate contained in about 20 mM Tris at about pH 8.0 for about 15 minutes at about 37°C.

9. A formulation comprising flea proteins that hydrolyze  $\alpha$ -naphthyl acetate  
5 to produce  $\alpha$ -naphthol, when said proteins are incubated in the presence of  $\alpha$ -naphthyl acetate contained in about 20 mM Tris at about pH 8.0 for about 15 minutes at about 37°C.

10. An isolated flea protein that hydrolyzes the methyl ester group of juvenile hormone to produce a juvenile hormone acid.

10 11. A formulation comprising flea proteins that hydrolyze the methyl ester group of juvenile hormone to produce a juvenile hormone acid.

12. A therapeutic composition that, when administered to an animal, reduces hematophagous ectoparasite infestation, said therapeutic composition comprising an excipient and a protective compound selected from the group consisting of: an isolated hematophagous ectoparasite carboxylesterase protein; a mimotope of an isolated hematophagous ectoparasite carboxylesterase protein; an isolated hematophagous ectoparasite carboxylesterase nucleic acid molecule that hybridizes under stringent hybridization conditions with a *Ctenocephalides felis* carboxylesterase gene; an isolated antibody that selectively binds to a hematophagous ectoparasite carboxylesterase protein; and an inhibitor of carboxylesterase activity identified by its ability to inhibit the activity of a flea carboxylesterase.

13. A method to reduce hematophagous ectoparasite infestation comprising treating an animal with a therapeutic composition comprising a protective compound selected from the group consisting of: an isolated hematophagous ectoparasite carboxylesterase protein; a mimotope of a hematophagous ectoparasite carboxylesterase protein; an isolated hematophagous ectoparasite carboxylesterase nucleic acid molecule that hybridizes under stringent hybridization conditions with a *Ctenocephalides felis* carboxylesterase gene; an isolated antibody that selectively binds to a hematophagous ectoparasite carboxylesterase protein; and an inhibitor of carboxylesterase activity identified by its ability to inhibit the activity of a flea carboxylesterase.

14. A method to produce a carboxylesterase protein, said method comprising culturing a cell capable of expressing said protein, said protein being encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a *Ctenocephalides felis* carboxylesterase gene.

5 15. A method to identify a compound capable of inhibiting flea carboxylesterase activity, said method comprising:

(a) contacting an isolated flea carboxylesterase with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has carboxylesterase activity; and

10 (b) determining if said putative inhibitory compound inhibits said activity.

16. A test kit to identify a compound capable of inhibiting flea carboxylesterase activity, said test kit comprising an isolated flea carboxylesterase protein having esterase activity and a means for determining the extent of inhibition of 15 said activity in the presence of a putative inhibitory compound.

17. The nucleic acid molecule of Claim 1, wherein said nucleic acid molecule is a flea nucleic acid molecule.

18. The nucleic acid molecule of Claim 1, wherein said nucleic acid molecule is selected from the group consisting of *Ctenocephalides*, *Ceratophyllus*, *Diamanus*, 20 *Echidnophaga*, *Nosopsyllus*, *Pulex*, *Tunga*, *Oropsylla*, *Orchopeus* and *Xenopsylla* nucleic acid molecules.

19. The nucleic acid molecule of Claims 1 or 2, wherein said nucleic acid molecule is selected from the group consisting of *Ctenocephalides felis*, *Ctenocephalides canis*, *Ceratophyllus pulicidae*, *Pulex irritans*, *Oropsylla (Thrassis) bacchi*, *Oropsylla (Diamanus) montana*, *Orchopeus howardi*, *Xenopsylla cheopis* and *Pulex simulans* nucleic acid molecules.

20. The nucleic acid molecule of Claims 1 or 2, wherein said nucleic acid molecule comprises a *Ctenocephalides felis* nucleic acid molecule.

21. The nucleic acid molecule of Claim 1, wherein said nucleic acid molecule 30 hybridizes under stringent hybridization conditions with a nucleic acid molecule selected

from the group consisting of nfE1<sub>401</sub>, nfE2<sub>364</sub>, nfE3<sub>421</sub>, nfE4<sub>524</sub>, nfE5<sub>1982</sub>, nfE5<sub>1515</sub>, nfE5<sub>2144</sub>, nfE5<sub>1650</sub>, nfE6<sub>1488</sub>, nfE6<sub>1792</sub>, nfE6<sub>1650</sub>, nfE7<sub>2836</sub>, nfE7<sub>1788</sub>, nfE7<sub>1710</sub>, nfE7<sub>650</sub>, nfE8<sub>2801</sub>, nfE8<sub>1785</sub>, nfE8<sub>1710</sub>, nfE9<sub>2007</sub>, nfE9<sub>1584</sub>, nfE9<sub>1540</sub>, nfE10<sub>1987</sub> and nfE10<sub>1590</sub>.

22. The nucleic acid molecule of Claim 1, wherein said nucleic acid molecule  
5 comprises a nucleic acid molecule selected from the group consisting of nfE1<sub>401</sub>, nfE2<sub>364</sub>, nfE3<sub>421</sub>, nfE4<sub>524</sub>, nfE5<sub>1982</sub>, nfE5<sub>1515</sub>, nfE5<sub>2144</sub>, nfE5<sub>1650</sub>, nfE6<sub>1488</sub>, nfE6<sub>1792</sub>, nfE6<sub>1650</sub>, nfE7<sub>2836</sub>, nfE7<sub>1788</sub>, nfE7<sub>1710</sub>, nfE7<sub>650</sub>, nfE8<sub>2801</sub>, nfE8<sub>1785</sub>, nfE8<sub>1710</sub>, nfE9<sub>2007</sub>, nfE9<sub>1584</sub>, nfE9<sub>1540</sub>, nfE10<sub>1987</sub> and nfE10<sub>1590</sub>.

23. The nucleic acid molecule of Claims 1 or 2, wherein said nucleic acid  
10 molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence that encodes a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:31, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:68, SEQ ID NO:73 and SEQ ID NO:74; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein having any of said amino acid sequences.

24. The nucleic acid molecule of Claims 1 or 2, wherein said nucleic acid  
20 molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:76, a nucleic acid molecule encoding a protein comprising amino acid sequence SEQ ID NO:74 SEQ ID NO:74; and a nucleic acid

molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

25. The nucleic acid molecule of Claim 1, wherein said nucleic acid molecule comprises an oligonucleotide.

5 26. A recombinant molecule comprising a nucleic acid molecule as set forth in Claims 1 or 2 operatively linked to a transcription control sequence.

27. A recombinant virus comprising a nucleic acid molecule as set forth in Claims 1 or 2.

28. A recombinant cell comprising a nucleic acid molecule as set forth in  
10 Claims 1 or 2.

29. The nucleic acid molecule of Claim 2, wherein said nucleic acid molecule comprises a nucleic acid sequence that encodes a carboxylesterase protein.

30. The nucleic acid molecule of Claim 2, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with the complement of a nucleic  
15 acid sequence encoding said protein.

31. The protein of Claim 4, wherein said protein, when administered to an animal, elicits an immune response against a carboxylesterase protein.

32. The protein of Claim 4, wherein said protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with nucleic acid  
20 molecule nfE6<sub>1792</sub>.

33. The protein of Claim 4, wherein said protein is selected from the group consisting of: a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:31, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:68, SEQ ID NO:73 and SEQ ID NO:74; and a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:31, SEQ ID NO:37, SEQ ID NO:39, SEQ ID

NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:68, SEQ ID NO:73 and SEQ ID NO:74.

34. An isolated antibody that selectively binds to a protein as set forth in  
5 Claim 4.

35. The formulation of Claim 6, wherein said proteins are selected from the group consisting of proteins having molecular weights ranging from about 60 kD to about 75 kD as determined by 14% Tris-glycine SDS-PAGE.

36. The formulation of Claim 6, wherein said proteins are isolated by the  
10 method comprising:

(a) applying soluble proteins of a flea extract to a p-aminobenzamidine agarose bead column;

(b) collecting unbound protein from said p-aminobenzamidine agarose bead column and applying said unbound protein to a gel filtration column;

15 (c) eluting proteins bound to said gel filtration column and applying said eluted proteins to a cation exchange chromatography column;

(d) eluting proteins bound to said cation exchange column and applying said eluted proteins to an anion exchange chromatography column; and

20 (e) eluting proteins bound to said anion exchange column with about 170 mM NaCl to obtain said flea carboxylesterase proteins.

37. The formulation of Claim 7, wherein said proteins are selected from the group consisting of proteins having pI values ranging from about pI 4.7 to about pI 5.2 as determined by IEF-PAGE.

38. The invention of Claims 8 or 9, wherein said incubation results in the  
25 production of from about 0.3 to at least about 2.5 absorbance units in the presence of Fast Blue when measured at 590 nm.

39. The protein of Claim 8, wherein said protein does not hydrolyze the methyl ester group of juvenile hormone.

40. The invention of Claims 8 or 9 or 10 or 11, wherein the mature form of  
30 said protein has a molecular weight ranging from about 60 kD to about 75 kD.

41. The invention of Claims 8 or 9, wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:19, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:53.

5 42. The invention of Claims 10 or 11, wherein hydrolyzation of said methyl ester group results in the release of at least about 120 counts per minute of  $^3\text{H}$ -juvenile hormone acid when determined by the method comprising:

(a) incubating said flea protein in the presence of  $^3\text{H}$ -juvenile hormone to create a reaction mixture;

10 (b) combining said reaction mixture with isoctane to produce an aqueous phase and an organic phase;

(c) recovering said aqueous phase; and

(d) determining the amount of  $^3\text{H}$ -juvenile hormone acid present in said aqueous phase.

15 43. The protein of Claim 10, wherein said protein does not hydrolyze  $\alpha$ -naphthyl acetate.

44. The invention of Claims 12 or 13, wherein said hematophagous ectoparasite carboxylesterase comprises a flea carboxylesterase.

20 45. The invention of Claims 12 or 13, wherein said hematophagous ectoparasite is a flea of a genus selected from the group consisting of *Ctenocephalides*, *Ceratophyllus*, *Diamanus*, *Echidnophaga*, *Nosopsyllus*, *Pulex*, *Tunga*, *Oropsylla*, *Orchopeus* and *Xenopsylla*.

25 46. The invention of Claims 12 or 13, wherein said hematophagous ectoparasite is a flea of a species selected from the group consisting of *Ctenocephalides felis*, *Ctenocephalides canis*, *Ceratophyllus pulicidae*, *Pulex irritans*, *Oropsylla (Thrassis) bacchi*, *Oropsylla (Diamanus) montana*, *Orchopeus howardi*, *Xenopsylla cheopis* and *Pulex simulans*.

47. The invention of Claims 12 or 13, wherein said hematophagous ectoparasite carboxylesterase comprises a juvenile hormone esterase.

48. The invention of Claims 12 or 13, wherein said composition further comprises a component selected from the group consisting of an adjuvant and a carrier.

49. The invention of Claims 12 or 13, wherein said composition further comprises a compound that reduces hematophagous ectoparasite burden by a method  
5 other than by reducing hematophagous ectoparasite carboxylesterase activity.

50. The invention of Claims 12 or 13, wherein said protective compound is selected from the group consisting of a naked nucleic acid vaccine, a recombinant virus vaccine and a recombinant cell vaccine.

51. The invention of Claims 12 or 13, wherein said inhibitor comprises a  
10 substrate analog of a hematophagous ectoparasite carboxylesterase.

52. The method of Claim 13, wherein said animal is selected from the group consisting of adult hematophagous ectoparasites, hematophagous ectoparasite larvae and animals susceptible to hematophagous ectoparasite infestation.

53. The method of Claim 13, wherein said animal is selected from the group  
15 consisting of adult fleas, flea larvae and animals susceptible to flea infestation.

54. The method of Claim 13, wherein hematophagous ectoparasite infestation is reduced by hematophagous ectoparasite larvae ingesting adult hematophagous ectoparasite feces comprising said therapeutic composition.

55. The method of Claim 13, wherein said adult hematophagous ectoparasite  
20 feces comprises anti-hematophagous ectoparasite carboxylesterase antibodies.

56. The method of Claim 13, wherein anti-hematophagous ectoparasite carboxylesterase antibodies are elicited in a host animal in response to administration of a reagent selected from the group consisting of one or more of said isolated  
25 hematophagous ectoparasite carboxylesterase proteins and one or more of said hematophagous ectoparasite carboxylesterase nucleic acid molecules, said adult hematophagous ectoparasite having fed from said host animal after said administration.

57. The method of Claim 13, wherein said animal is selected from the group consisting of mammals and birds.

58. The method of Claim 13, wherein said animal is selected from the group  
30 consisting of cats and dogs.

59. The method of Claim 14, wherein said cell expresses a nucleic acid molecule selected from the group consisting of pCro-nfE6<sub>1488</sub>, pTrc-nfE7<sub>650</sub>, pTrc-nfE7<sub>1710</sub>, pTrc-nfE8<sub>1710</sub>, pTrc-nfE5<sub>1650</sub>, pTrc-nfE9<sub>1540</sub>, pFB-nfE6<sub>1679</sub>, pVL-nfE7<sub>1802</sub>, pVL-fE8<sub>1792</sub> and pVL-nfE9<sub>1600</sub>.